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TEG PlateletMapping assay results may be misleading in the presence of cold stored platelets

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ABSTRACT

Background: Viscoelastic tests (VETs) are used widely to monitor hemostasis in settings such as cardiac surgery. There has also been renewed interest in cold stored platelets (CSP) to manage bleeding in this setting. CSPs are reported to have altered hemostatic properties compared to room temperature platelets (RTP), including activation of GPIIb/IIIa. We investigated whether the functional differences between CSP and RTP affected the performance of the PlateletMapping VET on the TEG 5000 and 6s analyzer.

Method: Platelet concentrates were divided equally into CSP (stored at 4°C±2°C) and RTP (stored at 22°C±2°C) fractions. Whole blood was treated to induce Platelet dysfunction (WBIPD) by incubating with anti-platelet drugs (1.0μM ticagrelor and 10μM aspirin) or by simulating cardiopulmonary bypass. WBIPD samples were then mixed with 20% by volume of CSP or RTP to model platelet transfusion before analysis using the PlateletMapping VET.

Results: Addition of CSP to WBIPD increased the PlateletMapping MA_{FIBRIN} and MA_{ADP} parameters with the TEG 5000 analyzer (both $p<0.0001$ compared to addition of buffer alone). This effect was not observed with RTP. The differential effect of CSP on the MA_{FIBRIN} corrected after pre-incubation with the GPIIb/IIIa antagonist tirofiban and was quantitatively less with the PlateletMapping test for the TEG 6s analyzer which contains the GPIIb/IIa antagonist abciximab.

Discussion: The PlateletMapping MA_{FIBRIN} and MA_{ADP} test results may be misleadingly high with CSP, particularly with the TEG 5000 analyzer, most likely due to constitutive activation of GPIIb/IIIa on CSP during storage. TEG PlateletMapping results should be interpreted with caution following CSP transfusion.

FIGURE LEGENDS

Figure 1: TEG PlateletMapping assay percentage (%) inhibition/aggregation is calculated from the maximal amplitude (MA) of three TEG assays; $MA_{THROMBIN}$ obtained using the standard TEG Kaolin activator, which stimulates thrombin generation in the blood sample. This parameter reflects the thrombin-dependent contributions of fibrin plus platelets to clot strength. MA_{FIBRIN} obtained using ActivatorF, containing reptilase and FXIII to generate and stabilize fibrin clot independently of thrombin. This reflects the fibrin component. $MA_{ADP/AA}$ in which ActivatorF is combined with the direct platelet activators adenosine di-phosphate (ADP) or arachidonic acid (AA). This reflects the contribution of platelet ADP or AA pathways to clot strength. The equation shown is used to calculate the '% aggregation' parameter from the three MA measurements and represents the contribution of either ADP-mediated or AA-mediated platelet activation to clot strength. Percentage (%) inhibition = 100 - % aggregation

Figure 2: TEG 5000 PlateletMapping assay maximal amplitude (MA) and calculated percentage (%) aggregation of; whole blood with induced thrombocytopathy (WBIPD) and addition of 20% v/v buffer alone, WBIPD with a simulated transfusion of 20% v/v, cold stored platelets (WBIPD + CSP), and WBIPD with a simulated transfusion of 20% v/v, room temperature stored platelets (WBIPD + RTP) (n = 14). A – $MA_{THROMBIN}$, B – MA_{FIBRIN}, C – MA_{ADP} and D – Calculated Percentage (%) aggregation. Statistical significance relative to WBIPD * = $p < 0.05$, ** = $p< 0.01$, *** = $p< 0.001$, **** = $p < 0.0001$, ns - non-significance.

Figure 3: TEG MA_{FIBRIN} dose response curve illustrating tirofiban treatment of pooled buffy coat CSP (n = 3) with the TEG 5000 analyzer, R2 = 0.93, 95% confidence intervals, standard error of the mean (SEM).

INTRODUCTION

The use of viscoelastic hemostatic tests (VETs) to guide resuscitation during major hemorrhage has increased significantly in recent years and is now recommended by the National Institute for Health and Care Excellence (NICE) for cardiac surgery.[1] VETs are also used commonly in other clinical settings such as traumatic or obstetric hemorrhage, but are not formally recommended in practice guidelines because evidence supporting clinical benefit remains incomplete.[2-5]

In both of the two most widely adopted VET technologies thromboelastography (TEG; Haemonetics, Braintree, MA) and rotational thromboelastometry (ROTEM; TEM International, Munich, Germany), clot formation is initiated using specific activators in whole blood samples housed in analysis cups with a suspended pin. Clot viscoelastic strength is then monitored continuously by detecting the mechanical resistance to oscillations applied to either cup (TEG) or pin (ROTEM).

Early versions of these VETs using contact (TEG-Kaolin test) or extrinsic pathway (ROTEM-EXTEM test) activators did not distinguish the specific contributions of either fibrin or platelets to clot strength. However, subsequent refinements of VETs utilize selective coagulation activators and platelet inhibitors to more precisely phenotype clot formation.[6,7] One example is the TEG PlateletMapping system which enables the platelet contribution to clot strength to be inferred from three different measurements of the maximum amplitude (MA) of the test traces:[8-10] 1. $MA_{THROMBIN}$ obtained using the standard TEG Kaolin activator, which stimulates thrombin generation in the blood sample. This parameter reflects the thrombin-dependent contributions of fibrin plus platelets to clot strength. 2. MA_{FIBRIN} obtained using ActivatorF, containing reptilase

and FXIII to generate and stabilize fibrin clot independently of thrombin. This reflects the fibrin component. 3. $MA_{ADP/AA}$ in which ActivatorF is combined with the direct platelet activators adenosine di-phosphate (ADP) or arachidonic acid (AA). This reflects the contribution of platelet ADP or AA pathways to clot strength. The main *'% inhibition/aggregation'* parameter from TEG PlateletMapping system is derived from the three MA measurements (Figure 1) and represents the contribution of either ADP-mediated or AA-mediated platelet activation to clot strength. This enables detection of the antiplatelet drugs aspirin (inhibits AA-mediated platelet function) and $P2Y_{12}$-blockers (inhibit ADP-mediated platelet function), which is critical in settings such as cardiac surgery to quantify the impact of anti-platelet drugs on clot strength and to guide platelet transfusion. Although initially developed for the TEG 5000 analyzer, the TEG PlateletMapping system has now been refined for the cartridge-based TEG 6s analyzer. For this analyzer, the ActivatorF reagent used to measure the MA_{FIBRIN} also includes the potent GPIIb/IIIa receptor antagonist abciximab.[10] This prevents platelet-fibrin interactions ensuring that the MA_{FIBRIN} only reflects the fibrin component of clot strength, thereby enabling a more reliable calculation of platelet *'percentage inhibition/aggregation'*.

One important trend in transfusion practice is the emergence of evidence that cold stored platelets (CSP) may be a useful alternative to conventional room temperature platelets (RTP) is settings such trauma or cardiac surgery.[11-15] It is established that CSP have altered expression of several surface adhesive receptors including GPIIb/IIIa, and also increased basal platelet activation compared to WSP.[16-19] Since these characteristics are critical for the endpoints of the TEG PlateletMapping test, we investigated whether CSP had a different effect on test results compared to RTP when

platelets were mixed with coagulopathic blood samples to simulate platelet transfusion.

MATERIALS AND METHODS

Preparation of platelet concentrates

Apheresis platelets were collected from healthy volunteers using the Trima Accel system (TerumoBCT Lakewood, CO) as previously described.[17,20-22] Platelets were collected as hyper-concentrated double collections, before being split equally into two platelet storage bags (Polyolefin PL-2410, Fenwal, Lake Zurich, IL) within two hours. Pooled buffy coat platelets were manufactured by NHS Blood and Transplant (NHSBT, Bristol UK) as previously described[23] in compliance with Blood Safety and Quality Regulations, Statutory Instrument 2005 No.50.[24] Both preparations were suspended in 35% plasma and 65% platelet additive solution (T-PAS, TerumoBCT, Lakewood, CO for apheresis and SSP+, MacoPharma, Mouvaux, France for buffy-coat platelets). Buffy-coat platelet concentrates were separated aseptically 24-36 hours after donation into two equal volumes into two TOTM bags (MacoPharma, Mouvaux, France) for storage at 22ºC ± 2 ºC with continuous agitation (RTP) or at 4ºC ± 2 ºC without agitation (CSP).

Preparation of Whole blood with induced platelet dysfunction (WBIPD) samples.

Whole blood (WB) was collected from healthy volunteers by peripheral venipuncture into 3.2% trisodium citrate tubes (BD Biosciences, Oxford, UK) in compliance with the Declaration of Helsinki. Two platelet dysfunction models were simulated and in this analysis are considered together (n = 14) 1.) *dual anti-platelet therapy:* incubation with 1.0μM ticagrelor and 10μM acetylsalicylic acid (ASA) for a minimum of 15 minutes (n

= 8 samples), and, 2) *extracorporeal bypass*; WB collected into a 500 mL Capiox reservoir (TerumoBCT, Lakewood, CO) containing 1 IU/mL heparin sulfate then circulated through an extracorporeal bypass circuit containing a roller pump (Cobe CV Model 43600, Sorin Biomedica, Milan, Italy), blood oxygenator (KIDS D100 Oxygenator, Sorin Biomedica, Milan, Italy) and pressure monitoring catheters (DLP catheters, 8 Fr, 12 Fr, Medtronic, Minneapolis, MN). Blood was circulated at a flow rate of 0.5 L/min with samples taken for analysis after 6 hours (n = 6 samples).

Mixing experiments and PlateletMapping analysis

Apheresis CSP or RTP stored between three and seven days were mixed with WBIPD samples at a dose of 20% by volume. In control experiments, the same volume of PlasmaLyte A pH 7.4 (Baxter, Deerfield, IL) was mixed with the WBIPD samples. The sample mixtures were analyzed using the TEG 5000 analyzer according to manufacturer's guidelines.[10] In additional experiments to study the effect of additional GPIIb/IIIa inhibition, buffy coat CSP were incubated with tirofiban (Correvio Pharma Corp. Vancouver, Canada) for at least 15 minutes and analyzed on both TEG 5000 and 6s platforms. Data were compared by analysis of variance (ANOVA) performed using GraphPad Prism version 8.2.1 (GraphPad Software, San Diego, CA).

RESULTS

Addition of both CSP and RTP increased the TEG 5000 $MA_{THROMBIN}$ in the WBIPD samples (both $p<0.001$ compared to addition of buffer alone, n = 14, figure 2A), consistent with reversal of thrombocytopathy. As expected, the TEG 5000 MA_{FIBRIN} was not altered by the addition of RTP to WBIPD. However, CSP substantially increased the MA_{FIBRIN} ($p<0.0001$ compared to addition of buffer alone, n = 14, figure

2B). There was a small, but non-significant increase in TEG 5000 MA_{ADP} with RTP, but a significant increase with CSP ($p<0.0001$ compared to addition of buffer alone, n = 14, figure 2C). The net effect of the differences in MA_{FIBRIN} and MA_{ADP} between RTP and CSP was an apparent reduction in the derived percentage aggregation parameter with CSP. However, because of the broad variation in results, this did not reach statistical significance (figure 2D).

One explanation for the increased MA_{FIBRIN} with CSP was that increased constitutive activation of GPIIb/IIIa could potentially cause the CSP to contribute to clot strength even though platelets are not directly activated with the ActivatorF reagent that is used to measure the $MA_{FIBRIN.}$ In order to test this, the CSP were incubated with the GPIIb/IIIa antagonist tirofiban before mixing with WBIPD and testing using the TEG 5000 platform. The addition of tirofiban resulted in a dose-dependent decrease in MA_{FIBRIN} with CSP, resulting in values similar to those with RTP at tirofiban concentrations above 1mM (figure 3). In order to assess whether the inclusion of abciximab in the MA_{FIBRIN} channel of the TEG 6s PlateletMapping cartridge reduces the CSP MA_{FIBRIN} in the same way as tirofiban, three samples of CSP products were analyzed on both TEG 5000 and 6s analyzers simultaneously. The MA_{FIBRIN} was 56.2 ± 3.28 mm (mean± SEM) and 45.2 ± 3.0 mm for TEG 5000 and TEG 6s respectively a reduction of 11 mm (19.6%) with TEG 6s.

Together, these observations support that the increased MA_{FIBRIN} with CSP compared to RTP was mostly mediated by activated GPIIb/IIIa on CSP and that this particularly impacts on the unmodified TEG 5000 PlateletMapping test because a GPIIb/IIIa antagonist is absent.

DISCUSSION

The TEG PlateletMapping assay has proved valuable in the management of patients, especially cardiac surgery patients in which there is frequently a complex thrombocytopathy that includes the effects of anti-platelet and the mechanical effect of cardiopulmonary bypass.[2,3,5] The calculation of percentage inhibition/aggregation with ADP or AA is essential to resolve the degree of platelet dysfunction. However, this derived parameter relies on the MA_{FIBRIN} being an accurate representation of the contribution of fibrin to clot strength which can then be subtracted from the $MA_{THROMBIN}$ and $MA_{ADP/AA}$ to yield the respective platelet contributions. Our results demonstrate that particularly with the TEG 5000 PlateletMapping test, this assumption may not be valid in the presence of CSP with which there is a dramatic increase in MA_{FIBRIN} compared with equivalent quantities of RTP. This differential effect was also observed with the MA_{ADP} result, which together resulted in a trend towards apparent underestimation of the percentage aggregation parameter in the presence of CSP.

We show further that the differential effect of CSP on the TEG 5000 PlateletMapping MA_{FIBRIN} can be ameliorated by the addition of the GPIIb/IIIa antagonist tirofiban. This observation is consistent with previous observations that cold storage of platelets results in constitutive activation of GPIIb/IIIa enabling binding of free fibrinogen in plasma, a process responsible for the formation of platelet clumps during cold storage in fibrinogen rich medium.[19] The bound fibrinogen may also complex for FXIII, providing a nucleation site for fibrin polymerisation.[17] We propose that addition of ActivatorF containing activated FXIII and reptilase for measurement of the PlateletMapping MA_{FIBRIN} and MA_{ADP}, results in crosslinked fibrin formation, but also recruitment of CSP to contribute to clot strength, even though they are not directly

activated by the test reagents. This effect is not observed with RTP because GPIIb/IIIa activation is minimal at room temperature storage. Consistent with this, the impact of CSP on MA_{FIBRIN} is less in the PlateletMapping test developed for the TEG 6s analyzer, because of the inclusion of the GPIIb/IIIa antagonist abciximab, although still present. The partial reduction in MA_{FIBRIN} in the TEG 6s with CSP may indicate that the dose of abciximab in the cartridge is insufficient. These findings indicate that the TEG 6s assay has the potential to be optimised further to mitigate against misleading results. In the meantime, our data suggest that caution should be exercised in interpreting TEG PlateletMapping results in the presence of CSP. Further validation, ideally in the *in vivo* setting is required to ensure patients are not put at risk.

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